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Correlation Between the Weight Loss Induced by Alkaline Ions and the Cationic Exchange Capacity of the *Nitella* Cell Wall

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ABSTRACT

There is a linear relationship between loss of weight and loss of cationic exchange capacity of *Nitella* cell walls, induced by treatment with alkaline ions. As the rate of *CEC* loss depends to a much greater extent on the ionic species than does the maximal loss quantity, it is suggested that the alkaline ions compete with the bivalent ions initially adsorbed for a particular type of site. These are different from the carboxylate exchange sites since the equivalent fractions of the alkaline ions adsorbed on to these sites are about the same for external concentrations where the effect of the different ions on weight and *CEC* loss is, nevertheless, quite different. Such sites give to the matrix pectin its structural coherence by preventing the action of electrostatic repulsive forces. In fact, it also appears that *CEC* loss is a linear function of the inverse of the square root of the internal ionic strength, a parameter proportional to the Debye length.

Key words: Cationic exchange capacity, cell wall, *Nitella flexilis*.

INTRODUCTION

The Characeae cell wall behaves like an excellent ion exchanger since it contains between 15% and 30% anhydrouronic acid, almost all the monomers of which are weak or non-esterified galacturonic acids. Between 60% and 65% of these uronic acids originate in the pectic substance, which is the major component of the amorphous structure of the matrix. The remainder is closely associated with hemicelluloses and cellulose microfibrils (Dainty, Hope, and Denby, 1960; Anderson and King, 1961*a*, *b*; Morikawa and Senda, 1974*a*). At physiological pH, the carboxyl groups of these uronic acids constitute so many exchange sites for the cations of the external solution. Their concentration, i.e. the cationic exchange capacity of the wall (*CEC*), was determined by different methods in *Nitella* or *Chara*. When the values of the *CEC* thus obtained are compared, it is found that a relation exists between the *CEC* and the nature of the pretreatment saline solutions (review in Gillet, 1989). Whereas the latter is between 1·1 and 1·3 eq. kg^{-1} dry weight when the walls analysed are taken from solutions still containing a certain proportion of bivalent ions, it drops to much lower values (0·5 to 0·7 eq. kg^{-1} dry weight) when the walls have undergone pretreatment with single monovalent ions. Also of note in this connection is the irreversible reduction of the *CEC* during potentiometric titration by acid solutions (Morikawa and Senda, 1974*a*) or the increased wall extensibility by acid pH and by certain monovalent and divalent cations (Morikawa and Senda, 1974*b*; Métraux and Taiz, 1977, 1979). These phenomena must be accompanied by a loss of pectic matter from the wall. Van Cutsem (1984), Van Cutsem and Gillet (1986) have, in fact, observed during ionic exchanges, when the quantity of bivalent ions in the wall is progressively reduced, that there is a loss of constitutive polysaccharides and a considerable decrease in weight when the concentration of bivalent ions in the wall is lowered to less than 0·35 eq. kg^{-1} dry weight. The results obtained in this study establish a linear relation between the weight loss induced in the isolated *Nitella* wall by the different alkaline ions and the corresponding lowering of *CEC*. They demonstrate that this loss of weight and of *CEC* depends less on the reduction of the quantity of bivalent ions

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in the external solution than on the intensity of ionic strength within the wall.

MATERIALS AND METHODS

Segments of cell wall several cm long were isolated from the internodal cell of *Nitella flexilis* (L.) Ag. by blowing out their contents. The walls were then cut longitudinally and washed thoroughly several times with a mixture of ethanol and diethyl ether to ensure that the cell membrane was completely removed. Following this treatment, the cell walls were equilibrated with renewed solutions of 10^{-2} N $MnCl_2$. Each wall was then cut into four segments. The first segment (joined with five others taken from five other walls treated in the same way), was used to determine the initial cationic exchange capacity (initial *CEC*) of the wall. The *CEC* is estimated by measuring, after extraction by HCl, the Mn^{2+} content of the wall segment by atomic absorption spectrophotometry. *CEC* was expressed on a dry weight basis which was recorded by a Cahn Model RG recording electrobalance. The other three segments (still joined together with five others in a batch) were respectively dipped in solutions of three different alkaline ions, each of the same concentration, and combined together in a different manner for each experiment. The concentration of the tested solutions varied from 5 to 500 mol m^{-3} in the different assays. For each assay, the treatment solutions were renewed seven times in 7 h. The segments of the cell walls were then individually blotted between two Whatman No. 3 filter papers and cut into two portions. One part was used to determine the wall content in the alkaline ion and in the remaining Mn^{2+}. The other was equilibrated in 10^{-2} N $MnCl_2$ solution with a view to measuring the final *CEC* and the final dry weight. This experimental procedure, with paired assays on the same cell wall, was used to minimize the individual cell wall fluctuations.

RESULTS

Treatment of the isolated *Nitella flexilis* wall with solutions of alkaline ions results in a loss of weight as well as a loss of ionic exchange capacity (*CEC*). Figure 1 shows, in the case of potassium chloride, that these losses increase rapidly with the concentration of the treatment solution when this does not exceed 50 mol m^{-3}. These losses are less extensive, however, when the external concentrations are higher than 100 mol m^{-3}.

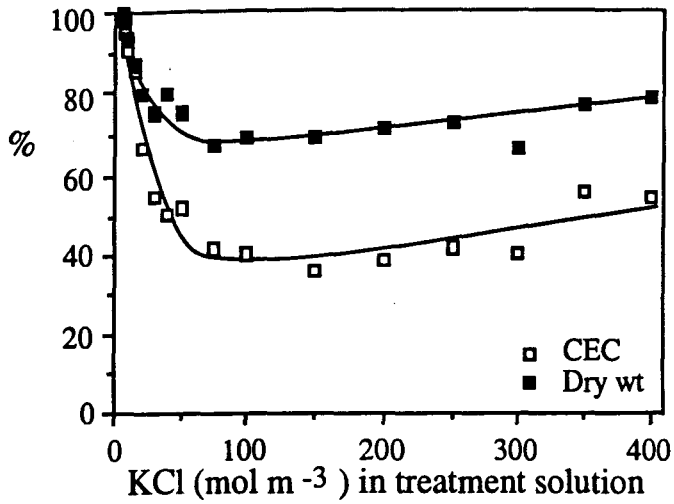

FIG. 1. Dry weight (as % initial weight) and cationic exchange capacity (as % initial *CEC*) of an isolated cell wall of *Nitella* as a function of the external KCl concentration.

As can be seen from Table 1, the maximum weight losses (*c.*33%) and *CEC* losses (*c.*67%) are fairly similar, whatever the treatment solution. But these results are obtained much more easily using solutions of lithium and caesium chloride than with sodium chloride solutions. Figure 2, where the differences between the loss of *CEC* obtained using solutions of K^+, Rb^+, Cs^+ and Li^+ as compared with that obtained with a solution of Na^+ are recorded for each concentration tested, shows clearly that the alkaline ions have very different effects on the range of concentrations between 10 mN and 30 mN. As we find the same type of differential effect on weight loss, it is not surprising that the loss of *CEC* is a linear function of the loss of weight (Fig. 3).

As well as causing a loss of weight and of *CEC*, the alkaline ions in increasing concentration tend to replace Mn^{2+} ions in the carboxylate sites previously saturated with these bivalent ions (cf. methods). As can be seen from Fig. 4A, the concentrations of adsorbed alkaline ions

TABLE 1. *Effects of the concentration and type of ion in the treatment solution on loss of weight and of* CEC *in the isolated* Nitella *wall*

The results are expressed as % of dry weight and as % of *CEC*, both measured before treatment. All values are the means of four replicates. The standard errors are between 3% and 7% for loss of weight and between 1% and 8% for loss of *CEC*.

External concentration (mol m^{-3})	% Dry weight					% *CEC*				
	Li	Na	K	Rb	Cs	Li	Na	K	Rb	Cs
5	97·8	100	100	99·6	97·0	95·8	100	100	94·1	92·3
7·5	86·8	100	98·6	96·4	86·8	80·2	100	94·9	96·0	77·8
10	75·2	97·3	92·6	86·7	75·2	60·1	98·9	88·1	69·6	53·1
15	70·4	94·6	87·2	77·5	67·9	47·5	95·7	85·4	69·6	43·7
20	68·7	92·8	75·2	70·7	69·8	36·7	87·4	58·2	49·5	37·2
30	64·7	69·2	71·7	66·6	65·2	33·0	43·1	44·8	38·0	30·7
100	66·7	66·2	68·9	66·8	65·2	31·6	34·1	35·5	32·6	29·2

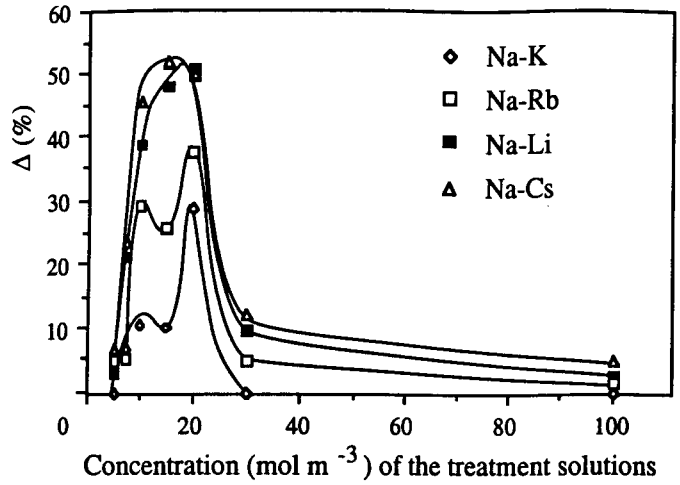

FIG. 2. Differences (Δ), for each external concentration tested, between the remaining percentage of *CEC* in the cell wall of *Nitella* after treatment with NaCl solutions and the remaining percentage of *CEC* after treatment with other alkaline ions (means of four replicates).

(eq. kg^{-1} dry weight) reach a maximum. This maximum is obtained for a concentration which is weakest when the ion considered causes maximal loss of *CEC*. However, these concentrations expressed in terms of the remaining *CEC* are not significantly different for the same external concentration from one monovalent ion to another (Fig. 4B). Thus the wall does not present, at first sight, a marked selectivity for one particular alkaline ion. The remaining concentration of bivalent ions rapidly decreases as the external concentration of monovalent ions increases. Their external equivalent fraction drops below 0·05 for an external concentration of alkaline ions equal to 30 mol m^{-3}. The difference between the total *CEC*, estimated according to the quantity of adsorbed Mn^{2+} ions after the walls have been again dipped into a solution of 10^{-2} N $MnCl_2$ following the treatment by monovalent ions, and the quantities of monovalent and bivalent ions adsorbed allows the total quantity of adsorbed protons and those recombined in the form of COOH groups to be calculated. The concentration of protons adsorbed is then determined on the basis of Donnan's theory (Briggs, Hope, and Robertson, 1961), taking 3·36 as the intrinsic *p*K value of the galacturonic acid (Van Cutsem and Gillet, 1983). Regardless of the treatment with alkaline ions, the concentration of adsorbed protons varies only very slightly and is very weak compared with that of the other adsorbed ions. The mean internal pH is 4·52, a value too high for the existence of an important pectic acid gel in the treated wall of *Nitella* (Jarvis, 1984) to be likely.

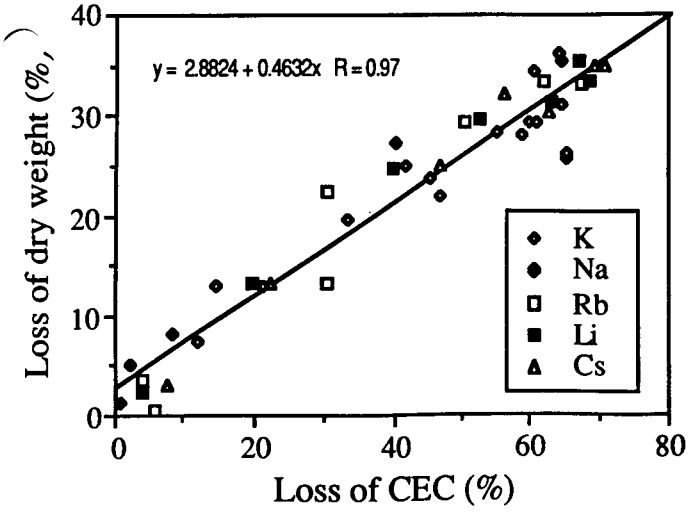

FIG. 3. Linear relationship between loss of dry weight (%) and loss of *CEC* (%) induced by treatment with different alkaline ions in the cell wall of *Nitella*. Each point is the mean of four replicates for each external concentration (from 5 to 400 mol m^{-3}) and for each alkaline ion (Cs^+, Rb^+, K^+, Na^+, Li^+).

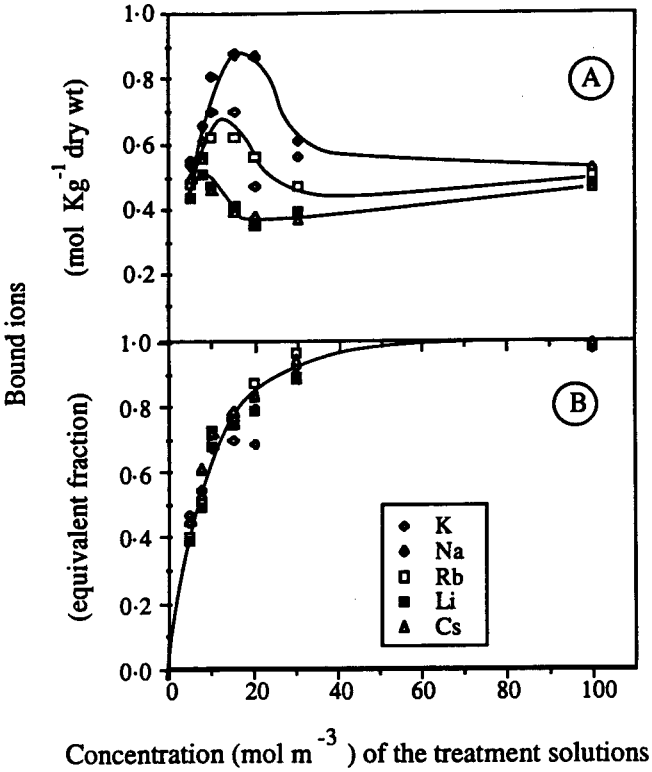

FIG. 4. Quantity of alkaline ions adsorbed in the cell wall of *Nitella* as a function of the external concentration. The same data are expressed as mol kg^{-1} dry weight in A and as equivalent fraction in the wall phase in B (Means of four replicates).

DISCUSSION

Origin of the pectic substances that were lost

The loss of *CEC* is due to a loss of uronic acids. As the molecular weight of a uronic acid in a polymerized chain is estimated to be 176 g and as the total *CEC* of a non-treated *Nitella* wall is 1·3 eq. kg^{-1} dry weight (review in Gillet, 1989), the maximum loss of *CEC* that was observed

(67%) should correspond to a maximum decrease of 20% of the dry weight. In this calculation, it is assumed that the uronic acids leave the wall in the form of chains of a pectic acid which contains 74% acid residues. (Anderson and King, 1961*c*). In fact, the maximum loss of weight is clearly greater since it reaches 33%. Treatment of the *Nitella* wall with solutions of alkaline ions, therefore, results in the elimination of both acid and other polymers. As it is unlikely that the linear relation between loss of *CEC* and weight loss passes through the origin ($P < 1\%$), it can thus be estimated, from Fig. 3, that among the neutral polymers released by the wall around 2% are released independently of the pectic acids (glycoproteins?, cf. Alary-Bernard, Briens, Quillet, and Goas, 1980) and 11% are released in association with the pectic acids (arabinogalactans?, cf. Anderson and King, 1961*c*). It is, moreover, interesting to compare the value of the maximum loss of uronic acids with the different quantities of uronic acids measured by Anderson and King (1961*a*, *b*) in the fractions of Characeae cell wall that can be considered as pectic. Their total uronic acid content amounts to 59% in *Nitella* and 67% in *Chara*. It is, therefore, possible that the 67% loss of *CEC* observed in our experiments is essentially due to a release of pectic substances which arise from the amorphous part of the matrix. Contrary to the observations of Morikawa and Senda (1974*a*), the bonds rendering pectic substances insoluble are not only acid-labile but also ion-labile.

Fixation sites of pectic substances

What is the nature of the sites which have an ordering effect on the pectinic molecules in the matrix of the *Nitella* wall? It does not appear that the carboxylic groups are essentially involved in the organization of these sites. Despite the fact that the alkaline ions, within the range of weak external concentrations, have very different effects on the loss of weight and of *CEC*, such variations are not found in the rate of their fixation as expressed in terms of the function of the total remaining *CEC* (Fig. 4). Their different effects do not result from clearly selective adsorption in the carboxylates sites. It is, therefore, necessary to postulate the existence of other bond sites responsible for the supra-molecular organization of the pectin. These might be ligand cavities (nestling sites) of different size (since Cs^+ and Li^+ have very similar effects) and markedly inferior in number to that of the carboxylic groups. These would be formed from neighbouring residues, not necessarily ionized, of pectic chains in appropriate conformation. They would behave like open chain cryptands or acyclic cryptands. By fixing bivalent ions, these cavities stabilize the architecture of pectic molecules (cf. review in Poonia and Bajaj, 1979). When the walls are treated with alkaline ions of increasing concentration, the latter start competing with bivalent ions which fulfil the role of substratum in this type of cryptand. The alkaline

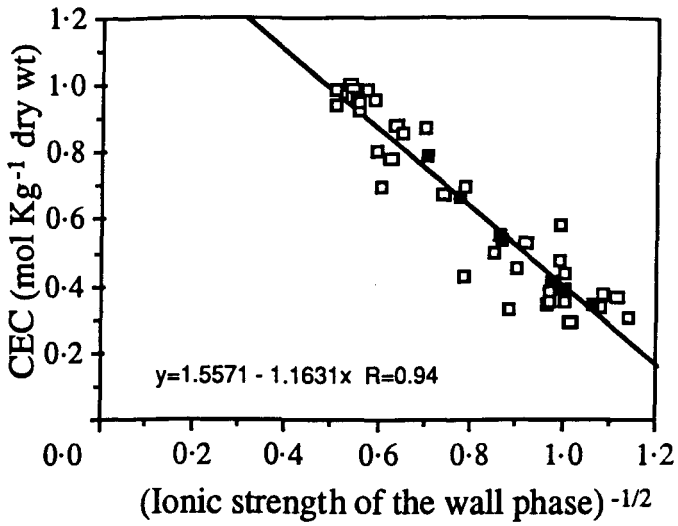

FIG. 5. Relationship between the quantity of remaining *CEC* and the intensity of the repulsive electrostatic forces in the cell wall phase of *Nitella*, estimated by the corresponding Debye length. Same data as in Fig. 3, but symbols □ are used for the results obtained in the external concentration range from 5 to 100 mol m^{-3} and symbols ■ for those obtained in the concentration range 150–400 mol m^{-3}.

ions whose spatial conformation most resembles that of these nestling sites most rapidly eliminate the bivalent ions. Our results show in fact, that the effects of the treatment by the alkaline ions depend on the type of ion in weak concentrations (10–30 mol m^{-3}) whereas they are the same in concentrations attaining 40 mol m^{-3} and beyond. A lowering in the stability of the ion–nestling site complex results from this exchange. The latter no longer succeed in preventing the action of repulsive electrostatic forces which normally operate between polymerized chains charged in the same manner. The loss of pectic acid and consequently of *CEC* ought then for each concentration to be proportional to the intensity of these forces. In Fig. 5, we have recorded the loss of *CEC* according to the inverse of the square root of the internal ionic strength, $I^{-1/2}$, calculated on the basis of experimental concentrations of adsorbed mono- and bivalent ions. This parameter is proportional to the Debye length and so proportional to the intensity of the repulsive electrostatic forces (Israelachvili, 1985). It can be seen that a linear relation does in fact exist in the concentration range from 5 to 100 mol m^{-3}, which leads to a progressively greater loss of *CEC*. If this interpretation of the results is correct, this relation must also exist in the concentration range from 250 to 500 mol m^{-3} which, nevertheless, results in progressively less loss of *CEC* (cf. Fig. 1). Figure 5 shows that the same relation holds for both ranges of concentrations despite their differing effects on *CEC* loss. The loss of weight and of *CEC* in the *Nitella* wall obtained by treatment with monovalent ions thus appears to be the result both of the elimination of particular fixation sites (which normally, by acting at the level of the matrix

polymers, give the wall its coherence) and of the effect of repulsive electrostatic forces which originate in the interaction of the electrical double layers surrounding the macromolecular chain. The existence of several types of site in the *Nitella* wall with or without a certain amount of overlapping has also been suggested by Morikawa, Tanizawa, and Senda (1974), by Métraux and Taiz (1979), and Van Cutsem, Gillet, Mestdagh, and Rouxhet (1985) to explain different effects on the orientation of the chains of pectic acids, on the extensibility of the wall, or the differential adsorption of Cu^{2+} ions.

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